Quantitative Insights into Gene Regulation

Robert G. Martin, Judah L. Rosner, National Institutes of Health;
Michael E. Wall, CCS-3

When environmental conditions change, cell survival can depend on the sudden production of proteins that are normally in low demand. Protein production is controlled by transcription factors that bind to DNA near genes and either increase or decrease RNA production. Many puzzles remain concerning the ways transcription factors do this.

There are hundreds of transcription factors in *Escherichia coli* and while most of these target only a small number of genes, there are several that regulate expression of 10 or more genes. Taken together, such global transcription factors directly regulate more than half of the ~4,300 genes in *E. coli,* and their regulatory interactions yield important insights into the organization of the genetic regulatory network [1-3]. Because they regulate so many genes, global transcription factors also play a large role in controlling cellular behavior; however, insights into behavior are currently limited by a lack of quantitative information about how transcription factors differentially regulate target genes.

We are performing quantitative studies of gene regulation by the global transcription factor MarA in *E. coli.* MarA activates ~40 genes of the E. coli chromosome, resulting in different levels of resistance to a wide array of antibiotics and superoxides (see [4] for references). We placed the expression of MarA under the control of an external signal and examined the expression of 10 target genes as a function of activator concentration [5]. We found that activation of different genes occurs in a well-defined order with respect to the level of MarA, enabling cells to mount a response that is commensurate to the level of threat detected in the environment. In contrast with a commonly held assumption, we found that the order of activation does not parallel the strength of MarA binding to DNA near the target gene. This finding suggested that interactions between MarA and the RNA polymerase transcriptional machinery play an important role in determining the order of activation, but the data did not immediately reveal what the nature of these interactions might be.

Next, we developed a computational model of gene regulation to understand how interactions between MarA and polymerase activate transcription of *marRAB, sodA,* and *micF* [6]—of the 10 targets we examined previously, these three are the only ones that exhibited saturation at high MarA, which provided an important constraint for the modeling. The model was specifically designed to compare the textbook model of recruitment, in which MarA increases polymerase binding but does not increase the rate of post-binding events [7,8], with a more general model in which activator can either increase or decrease polymerase binding, and can either increase or decrease the rate of post-binding events (Fig. 1). The model clearly explains the lack of correspondence between the order of activation and the MarA-DNA affinity, and indicates that the order of activation can only be predicted using information about the strength of the full MarA-polymerase-DNA interaction. Instead of favoring activation by recruitment, the modeling favors activation by increasing the likelihood that polymerase will initiate transcription when it is already bound to DNA near the target gene (Fig. 2). It also suggests that MarA can activate expression while decreasing the overall presence of the transcription machinery at the start of a gene. This mechanism is opposite to the textbook model of recruitment; nevertheless it enables cells to respond quickly to environmental challenges and is likely of general importance for gene regulation in *E. coli* and beyond.

For more information contact Michael E. Wall at mewall@lanl.gov.

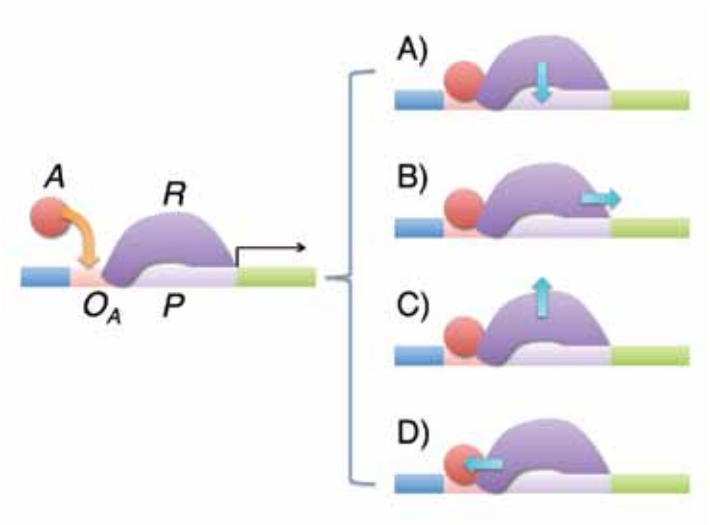

Fig. 1. Illustration of ways that bacteria might control mRNA transcription. A) In the textbook model of activation by recruitment, an activator (A) binds to an operator sequence (O_A) and increases the presence of RNA polymerase (R) at the start site (P). B) Although usually ignored, it has long been known that activators can accelerate the initiation step when polymerase is bound. C) Wall et al. [6] found that activation can involve a decrease in the presence of polymerase at the start site; although this effect is naturally associated with B), it was previously ignored. D) Activator can also retard the initiation step, a neglected effect that is naturally associated with the textbook model.

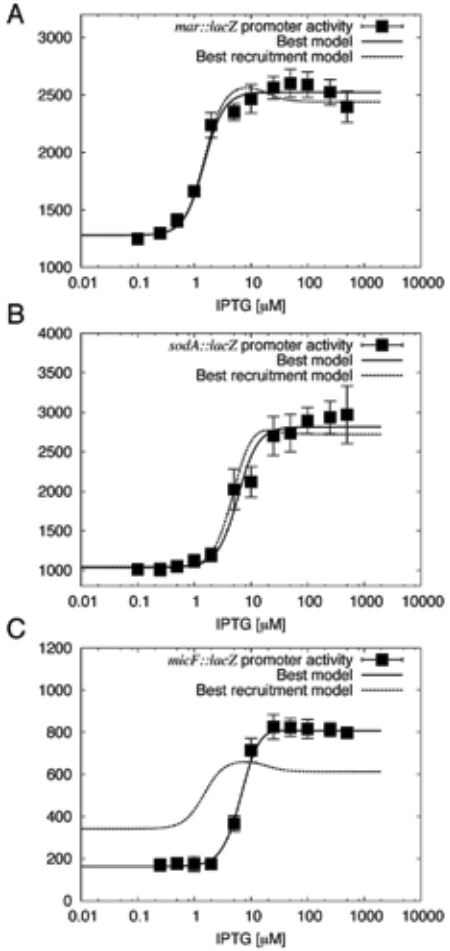

Fig.2. Comparison of alternative models of transcriptional activation. A) For activation of* marRAB *the fit of the recruitment model is reasonable (Fig. 1A), but the fit of models involving acceleration of the initiation step is better (Fig. 1B). B) For activation of* sodA*, the best models involve not only acceleration (Fig. 1B), but also a decrease in the presence of polymerase at the start site (Fig. 1C). C) Activation of* micF *is poorly fit by the recruitment model, but is well fit by models that involve both acceleration of the initiation step (Fig. 1B) and a decrease in polymerase binding (Fig. 1C).

[1] J.A. Freyre-Gonzalez et al., *Genome Biol.* **9,** R154 (2008).
[2] A. Martinez-Antonio, *J. Collado-Vides, Curr. Opin. Microbiol.* **6,** 482 (2003).
[3] S.S. Shen-Orr et al., *Nat. Genet.* **31,** 64 (2002).
[4] D.G. White, M.N. Alekshun, P.F. McDermott, *Frontiers in Antimicrobial Resistance,* ASM Press (2005).
[5] R. G. Martin et al., *J. Mol. Biol.* **380,** 278 (2008).
[6]M.E. Wall et al., *PLoS Computational Biology* **5,** e1000614 (2009).
[7] M. Ptashne, *Philos. Transact. A Math. Phys. Eng. Sci.* **361,** 1223 (2003).
[8] M. Ptashne, A. Gann, *Genes & Signals,* Cold Spring Harbor Press (2002).

Funding Acknowledgments
- LANL Directed Research and Development Program
- National Institutes of Health (NIH)–Intramural Research Program